NAVAL HEALTH RESEARCH CENTER

The Emerging Role of Vitamin D in Cancer Risk Reduction

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The Emerging Role of Vitamin D in Cancer Risk Reduction

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List of abbreviations

Abbreviation	Description
25(OH)D	Calcifediol, the main form in which vitamin D is stored in the body. It is a product of enzymatic hydroxylation of vitamin D in the liver. Also known as 25-hydroxyvitamin D or calcidiol. If the source of the basic molecule is known, it may be identified with a subscript at the end (see vitamin D_2 and vitamin D_3, below).
1,25$(OH)_2$D	Calcitriol, the most potent form of vitamin D in terms of calcitropic effects. This compound is also known as 1,25-vitamin D, 1alpha-25-vitamin D or 1alpha-25$(OH)_2$D. If the source of the basic molecule is known, it may be identified with a subscript.
Apc (min)	Adenomatous polyposis coli (min) mice
DAG	Diacylglycerol
EB 1089	22,24-diene-24a,26a,27a-trihomo-1,25$(OH)_2$D, a vitamin D analog
ER	Endoplasmic reticulum
IGF-I	Insulin-like growth factor I
IP3	Inositol 1,4,5-triphosphate
NHANES I	National Health and Nutrition Examination Survey Epidemiologic follow-up study, 1971-1975 to 1992.
PKC	Protein kinase C
RO 23-7553	1,25-dihydroxy-16-ene-23-yne-cholecalciferol.
RO 24-5531	1 alpha,25-Dihydroxy-16-ene-23-yne-26,27-hexafluoro-cholecalciferol
RO 25-6760	1-alpha,25-dihydroxy-16-ene-23yne-26,27-hexafluoro-19-nor-cholecalciferol
RO 27-0574	A 20-cyclopropyl analog of 1,25$(OH)_2$ vitamin D_3
TGF-a	Transforming growth factor alpha
VDR	Vitamin D receptor
Vitamin D_2	Ergocalciferol – Vitamin D derived from plant sources (yeast).
Vitamin D_3	Cholecalciferol – Vitamin D synthesized in the skin when ultraviolet B energy is absorbed.

Abstract

Recent advances confirming a role of vitamin D in reduction of the risk of cancer have created a new field of clinical interest. This is a review of the laboratory, clinical, and epidemiological evidence supporting the role of vitamin D in reduction of the incidence of breast, colon and prostate cancer, and the biological mechanisms of vitamin D anticarcinogenesis. It describes the prevalence of vitamin D deficiency in the United States and provides guidelines for clinical testing and interventions.

Introduction

Recent advances confirming a role of vitamin D in prevention of cancer have created a new field of clinical interest (1-15). While there is persistent interest in vitamin D for its role in reducing risk of fractures (15-20), its newly defined nonskeletal effects, including a contribution to reducing the risk of several forms of cancer, recently are becoming more widely known (21-24).

Mechanisms of Vitamin D Anticarcinogenesis

A wide variety of tissues not directly associated with calcium metabolism have vitamin D nuclear receptors (VDRs). These include tissues of the breast (25), colon (26-28), skin (melanocytes) (29), pituitary (30, 31) and CD4/CD8 lymphocytes (32, 33). The first insight into the function of $1,25(OH)_2D_3$ (calcitriol) in tissues not directly related to calcium metabolism was the observation that when leukemic cells having a VDR were incubated with $1,25(OH)_2D_3$, their proliferation was inhibited and they matured into granulocytes (34). It was soon learned that $1,25(OH)_2D_3$ had functions in regulation of cell growth and promotion of cell maturation in experimental models of cancer (13, 35-40), and in treatment of the hyperproliferative skin disorder psoriasis (41).

Epidemiological Evidence: Latitude, Vitamin D, and Cancer

In the same time period, several epidemiologic studies demonstrated higher mortality rates of cancer of the colon (42-44), breast (43, 44), prostate (43-45), and ovary (43, 44, 46) in the northeastern sector of the United States, compared with considerably lower rates in the southern tier of states. Dietary differences could not account for this regional difference (42). While other differences may play a role (47, 48), it has been established that the northern tier of states receives less sunlight annually than the southern tier, mainly due to the longer duration and severity of winter in the north, and to far lower levels of ultraviolet B (UVB) radiation during fall through spring in the northern United States (49-52). There is a sharp threshold for biosynthesis of vitamin D in human skin (53, 54) that occurs at an intensity of UVB radiation that is reached in the southern states of the United States, but not the northern states, Canada, and Europe, during November through March (54). High age-adjusted mortality rates of female breast cancer also have been reported in areas of low winter UVB in the Northeast, where acid haze, a form of air pollution that diminishes atmospheric transmission of UVB, is a factor (87).

Similarly high rates have been reported in areas of low winter UVB in the former USSR (55). An association of low regional sunlight level with risk of colon and breast cancer also has been reported in migrant studies (56-58). The inverse association of colon, breast, prostate, and other cancers with sunlight was recently confirmed at the population level in the United States (44).

Once it was recognized that normal colon, breast, prostate and other epithelial cells have VDRs and that UVB exposure increases circulating 25(OH)D (calcifediol) levels in a dose-response manner (59), an explanation accounting for the unusual geographic distribution emerged. Increased exposure to sunlight increases the amount of circulating $25(OH)D_3$ substrate, which supports increased synthesis in tissues of $1,25(OH)_2D_3$ that regulates cell proliferation (35, 36, 60-62). However, increasing exposure to UVB usually does not result in marked elevation in circulating levels of $1,25(OH)_2D_3$, since the synthesis of $1,25(OH)_2D_3$ is tightly regulated in the kidney by serum levels of calcium, phosphorus, and parathyroid hormone (59). An important exception is individuals whose vitamin D synthesis and intake is very low, including young adults during winter months in temperate climates (63, 64). Vitamin D deficiency also is especially prevalent in older adults (65), who tend to have very low levels of 25(OH)D and $1,25(OH)_2D$ during the winter months in New England, the Mid-Atlantic states, and Europe (66). Sunscreen usage, which is self-reported by about half the population in the United States (67), also contributes to vitamin D deficiency; use of a sunscreen with a protection factor of 8 reduces vitamin D synthesis by 97.5% (68).

It soon was recognized that tissues other than the kidney had the enzymatic ability to synthesize $1,25(OH)_2D$ from the circulating substrate, 25(OH)D. It is now known that normal and malignant colon (69), prostate cells (70), keratinocytes (71, 72), lymph nodes (granulocytes) (72), pancreatic islet cells (72), cerebral cortex and cerebellar tissue cells (72), and testicular Leydig cells (72) express the mitochondrial 25(OH)D-1alpha-OHase enzyme that can convert $25(OH)D_3$ to $1,25(OH)_2D_3$ (72). This was an enormously important advance that helps to explain the influence of vitamin D from sunlight and other sources on risk of cancer. The human gene for the 1alpha-OHase enzyme has been cloned and localized to chromosome 12 (73). Mutations of this gene, known as the CYP 1 alpha gene, are the cause of vitamin D-dependent rickets, type 1 (73, 74). The 1alpha-OHase is not regulated by parathyroid hormone in tissues other than the kidney, and the 1alpha-OHase gene promoter region is regulated differently in the kidney than other tissues (75). This potent antiproliferative hormone, in turn, regulates cell growth by maintaining cells in the G0/G1 phase of the cell cycle, for example, in

prostate cancer cell lines (76). It also maintains normal cellular differentiation in several other tissues (77, 78), and indirectly provides a pathway for apoptosis, or programmed cellular death (78, 79).

Exposure to UVB radiation from sunlight (80, 81) or oral vitamin D intake (81, 82) increases the circulating level of 25(OH)D, which, in turn, provides a higher concentration of substrate available for the intracellular synthesis of $1,25(OH)_2D$ in a wide range of tissues. This is an important finding that accounts for observations that high vitamin D status and residence at sunny latitudes are associated with lower mortality rates from cancer of the colon (42, 43, 83-85), breast (5, 6, 43, 86, 87), ovary (43, 46), and prostate (43, 45, 88-90).

Mechanisms of Vitamin D and Calcium in Reduction of Risk of Colon Cancer

A key mechanism of the antineoplastic effect of vitamin D on colon cancer was elucidated by Davies and associates (91). Advances in fluorescent intracellular dye technology (92, 93) revealed that certain normally occurring pulses in the intracellular calcium concentration are needed to induce differentiation of intestinal epithelial cells, and to induce apoptosis in these cells (91). Normal human colonocytes have nuclear receptors for $1,25(OH)_2D$ (26) and produce calcium-binding proteins known as calbindins, which are used for temporary storage and release of calcium ions in the cytoplasm (97)(Fig. 1). Calbindin 28kD, the most common form, is a 261-amino protein coded by chromosome 8 (8q21.3-q22.1) in response to various stimuli, including vitamin D metabolites. Each calbindin molecule reversibly binds calcium ions. Calbindin molecules are storage depots for calcium in the cytoplasm that can rapidly release calcium to the cytoplasm if signaled to do so.

Colonocytes also contain calcium channels or similar structures, including a vitamin D-dependent channel-like intestinal and colonic transport protein (CaT1), which has been cloned (94). It is homologous to the kidney epithelial apical calcium channel (EcaC) (95). There is also loose binding of calcium ions to the endoplasmic reticulum (96).

Colonocytes are organized into invaginations of the mucosa known as crypts (Fig. 2) (91, 98, 99). Epithelial cells arise from the base of each crypt and migrate to the mouth of the crypt, where they undergo programmed death (91). When the colonocyte reaches the mouth of the crypt in the normal colon, the intracellular calcium pulses to a level that is lethal to the cell. The gradual increase in intracellular calcium as the colonocyte rises through and matures in the crypt is known as the intracellular calcium gradient (Fig. 3)(100). Such a gradient also has been

reported in the epidermis, where the intracellular calcium concentration is low at the dermal-epidermal junction but rises in more distal layers, reaching a peak near the proximal layer of the stratum corneum, immediately preceding apoptosis of keratinocytes into dead squames in the stratum corneum (101, 102).

When a colonocyte reaches the mouth of the crypt in the vitamin D-replete individual, calcium-mediated apoptosis occurs and the cell dies (91). Deficiency of vitamin D results in loss of the intracellular calcium gradient in the crypt architecture (Fig. 4). This loss interferes with calcium-mediated terminal differentiation of colonocytes and apoptosis of colonocytes at the mouths of the crypts (91). This loss allows aged and mutated intestinal epithelial cells to persist and accumulate for abnormally long periods of time (91, 103, 104).

Highly proliferative colonocyte populations have been documented in individuals with a family history of colon cancer (105-107). Intracellular calcium pulses of sufficient amplitude to induce immediate cell death occur only in the vitamin D-replete state (91). Pulsing of calcium is dependent on the presence of calbindin, which reversibly binds calcium and releases it in a pulsatile fashion when needed for terminal differentiation and apoptosis (91, 108). Since vitamin D is required for synthesis of calbindin, its deficiency results in destruction of the pulsatile release of intracellular ionized calcium (91), preventing apoptosis.

Lamprecht and Lipkin (109) recently critically discussed mechanisms in calcium and vitamin D inhibition of colorectal carcinogenesis involving genomic and non-genomic pathways (109). A vitamin D metabolite-activated nongenomic receptor located in the plasma membrane induces a rapid Ca^{2+} response through a G-protein-coupled Ca^{2+} membrane channel (109-111). This response sets in motion a cascade of intracellular events, including Ca^{2+} activation of phosphoinositide-specific phospholipase C, resulting in the formation of two intracellular messengers, inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG). IP3 opens ER-bound channels, thus releasing stored Ca^{2+} while DAG activates protein kinase C (PKC) in the plasma membrane. Activation of specific PKC isoenzymes induces differentiation in intestinal cells (109). This cascade of biochemical events is also observed following challenge of cells of different lineages with extracellular calcium. Considerable evidence is available showing that extracellular calcium acts via a calcium-sensing receptor, a typical G-protein receptor present in the gastrointestinal tract (109).

Genomic actions of vitamin D metabolites via the nuclear VDR are well established (109). The modes of action of the secosteroids acting at various levels of cellular organization and function

in restraining growth and inducing differentiation in intestinal and colonic cells have been reviewed recently (109).

Further evidence for a predominant role of vitamin D in inhibiting development of colon cancer is that VDR expression remains sustained in low-grade adenomas, but declines to lower levels in poorly differentiated tumors exhibiting a malignant phenotype (112, 113). Consistent with these and related laboratory findings, the rate of colonic epithelial cell proliferation in humans is low when the serum 25(OH) D level is high, and high when serum 25(OH)D is low, with a dose-response gradient (98).

Vitamin D Levels and Colon Cancer: Epidemiologic Studies

It has been known for some time that colon cancer death rates tend to be low in sunny areas and high in areas of with low winter sunlight levels, particularly in the industrialized Northeast (Fig. 5). A nested case-control study designed to determine whether vitamin D might explain this geographic gradient was performed of 25,620 volunteers who provided blood samples (84). The probable association of low serum 25(OH)D with risk of colon cancer incidence was confirmed. Individuals with circulating 25(OH)D levels less than 20 ng/ml had twice the incidence rate of colon cancer during the 8-year study follow-up period as those with lower levels (84). Most of the association of serum 25(OH)D with risk of colon cancer occurred during the first decade of follow-up, suggesting that the main effect of vitamin D is during the promotional phase (114). Several epidemiological studies have reported higher risk of colon cancer in individuals who consume low amounts of vitamin D, including the Western Electric Cohort (115), the Harvard Nurses' Health Cohort (116), the Harvard Male Health Professionals Cohort (117), the Iowa Women's Health Cohort (118), and studies of residents of Stockholm (119) and Wisconsin (120). One additional study reported higher risk of colon cancer (121) and another cohort study found a higher incidence of rectal cancer in association with low intake of vitamin D, but those studies did not acheive criteria for statistical significance (122). The cohort study of rectal cancer found that individuals in the lowest third of the population for intake of vitamin D and calcium had approximately twice the incidence rate of rectal cancer as those in the highest third, indicating a synergistic effect of vitamin D and calcium intake (122). A scattering of case-control studies performed in southern Europe or other sunny climates did not find an effect of dietary vitamin D on risk, possibly because of uniformly high serum 25(OH)D levels in residents of those areas due to high solar UVB exposure (123-125).

Women whose plasma $1,25(OH)_2D$ concentration was slightly below average also had a 60% increased risk of distal colorectal adenomas (126). The relationship was stronger for large or villous adenomas, and among women with consistent vitamin D intake over the 10 years prior to collection of the blood sample. Compared with those in the lowest quartile of plasma 25(OH)D, women in the second quartile (odds ratio [OR], 0.64; 95% confidence interval [CI], 0.41-1.00) and third quartile (OR, 0.58; 95% CI, 0.36-0.95) were at lower risk (126). There was no reduction in risk associated with the top quartile.

This inverse association of serum vitamin D metabolites with risk of colon cancer could be related to the ability of $1,25(OH)_2D_3$ to block the stimulatory effect of epidermal growth factor on colon epithelial cells (127). It might also account for the approximately 40% lower risk of colon cancer in women taking estrogen, compared with placebo, that recently was observed in the Women's Health Initiative Clinical Trial (130). Exogenous estrogen increases the vitamin D receptor concentration in the bowel (128). It also upregulates serum $1,25(OH)_2D$ in women with abnormally low levels (129). The favorable effect of estrogen usage on colon cancer risk was even greater than the 34% reduction in incidence of hip fractures that occurred in the women taking estrogen (130). Consistent with this finding, Framingham women with high bone mass (in the top third of the cohort) had significantly reduced incidence rates of colon cancer (multiple-adjusted relative risk (RR) = 0.4) (131). Intake of milk (which contains 100 IU of vitamin D per 250-ml glass) and dairy products was associated with lower than average risk of colorectal cancer in women in the New York University Women's Health Study cohort (132), with a similar favorable association for calcium. Findings nearly identical to these were reported in two case-control studies (133, 134).

Adults who took a daily multivitamin supplement containing 400 IU of vitamin D (135) had approximately half the risk of colon cancer as those who did not, according to a large case-control study based on Surveillance, Epidemiology and End Results program cases and population controls (135). High dietary calcium intake was associated with reduced risk of adenomatous polyps (136), an effect similar to that of high serum 25(OH)D and $1,25(OH)_2D$ (126). Several epidemiological studies have reported lower risk of colon cancer in those with high levels of physical activity (137-140), which possibly could be explained by the increase in serum $1,25(OH)_2D$ concentration that results from exercise and physical activity (141, 142).

Mechanism of Vitamin D in Breast Cancer

Terminal ductal lobular units, the units of epithelial organization of the breast, have features that are analogous to intestinal crypts, such as a monolayer or bilayer of epithelial cells that originate at or near the base of the terminal duct and move distally toward the mouth of the duct (22), and the presence of vitamin D receptors (25, 143). Breast epithelial cells terminally differentiate (144, 145) in response to the high levels of circulating $1,25(OH)_2D$ that are characteristic of late pregnancy (146-148) and early lactation (149).

When vitamin D intake or synthesis is high, even in the absence of pregnancy, breast epithelial cells terminally differentiate and undergo apoptosis at the mouth of the ductal units (144, 145), preventing crowding of epithelial cells at the mouths of ducts. When intake of vitamin D is low, apoptosis is considerably reduced (79, 145) and crowding occurs. The average number of epithelial cells in the terminal ducts is much greater in animals on a diet deficient in vitamin D than on a diet that is replete in vitamin D (22, 144, 145). Animals consuming low amounts of vitamin D experience reduplication of terminal ducts, with approximately twice the number of ducts as those consuming the normal intake (22, 144, 145). This mechanism, which involves the $1,25(OH)_2D$ receptor, also occurs in malignant breast cancer cell lines (150).

Occurrence of mammary tumors was reduced by four fifths in animals receiving adequate vitamin D compared with those receiving the minimal dose necessary for normal growth (2, 22, 144). Vitamin D also had a larger independent effect on risk of mammary carcinogenesis than high intake of fat or deficient intake of calcium (2, 22, 144). The effect of vitamin D on mammary carcinogenesis was particularly strong early in life, suggesting that low circulating levels of 25(OH)D in adolescence may be important contributors to later breast cancer risk (144). The roles of vitamin D, calcium, and fats may be linked (2, 22, 144). High levels of $1,25(OH)_2D$ are also associated with slower progression of metastatic breast cancer (151).

Epidemiological Studies of Vitamin D and Reduction in Risk of Breast Cancer

A recent study using prediagnostic serum from cancer cases and healthy controls reported that white women in the lowest quartile of serum $1,25(OH)_2D$ had five times the risk of breast cancer as those in the highest quartile (OR, 5.2, 95% CI, 2.1-12.8) (152). A prospective study of the NHANESI cohort reported lower incidence rates of breast cancer in women regularly

exposed to sunlight in the sunniest regions of the United States, and in those who consumed above-average amounts of vitamin D (6).

Role of Vitamin D in Ovarian Cancer

$1,25(OH)_2D$ inhibits the growth of ovarian cancer cells in tissue culture (153). It counteracts the growth-stimulatory effect of dihydrotesterone (154) and downregulates the c-myc proto-oncogene (155). These findings are supported by epidemiological data indicating a lower mortality rate of the disease in sunny areas of the United States in perimenopausal women (46). $1,25(OH)_2D$ inhibits growth of ovarian cancer in the OVCAR-3 cell line, where it opposes the growth-stimulatory effect of androgens (154). The effect of vitamin D metabolites occurs early in the process of ovarian carcinogenesis, so administration of $1,25(OH)_2D$ to women with the disease does not reduce the rate of progression (156).

Mechanisms and Clinical Effects of Vitamin D in the Prostate

Vitamin D is involved in normal differentiation of the prostate (21), vitamin D receptors are expressed in the prostatic epithelium (39, 157, 158) and malignant prostatic cells (159-161), and prostatic cells are able to convert $25(OH)_2D$ to $1,25(OH)_2D$ (70). Inhibition of proliferation of prostate cancer cells in tissue culture occurs with exposure to either $1,25(OH)_2D$ (70) or 25(OH)D (39, 70). $1,25\ (OH)_2D$ also inhibits the invasiveness of human prostate cancer cells (21, 162) and cell adhesion and migration to the basement membrane matrix protein, laminin (162). Liarozole, an inhibitor of $1,25\text{-}(OH)_2D_3$-24-hydroxylase makes $1,25(OH)_2D$ persist longer and enhances its antiproliferative effect, even in highly resistant cell lines (163). Transfection of the gene for the 1alpha-OHase enzyme into prostate cancer cells that have lost it reduces their growth rate (164).

In a cohort study of 19,000 men followed for 13 years, men whose 25(OH)D level was below the median had 1.7 times the incidence of prostate cancer as those above the median (89). Among younger men (under age 57 years) those with low 25(OH)D levels had 3.5 times the incidence of prostate cancer and 6.3 times the incidence of cancer that had spread beyond the prostatic capsule (89).

In a nested case-control study of stored serum based on 250,000 samples collected from members of the Kaiser Foundation Health Plan, white and black men who had lower than average levels of $1,25(OH)_2D$ had higher risk of prostate cancer (165). Men in the lowest

quarter of circulating $1,25(OH)_2D$ had 20% higher incidence of prostate cancer than those in the highest quarter (165, 166). The influence of $1,25(OH)_2D$ was greatest in men with low serum 25(OH)D (166). A smaller nested case-control study found no association (167). Another small nested case-control study of Hawaiian Japanese men reported 20% higher risk of prostate cancer in men in the lowest quartile of serum 25(OH)D, although the trend did not achieve statistical significance, probably due to limited sample size.

Age-adjusted, race-specific prostate cancer mortality rates are generally higher in northern counties of the United States and lowest in sunnier southern counties (43, 44, 168), consistent with these laboratory and clinical findings. Men with a history of exposure to high levels of solar ultraviolet radiation are at lower than average risk of prostate cancer (169, 170). In one study (89), prostate cancer risk was highest among the group of younger men (ages 40-51 years) with low serum 25(OH)D. However low serum 25(OH)D appeared not to be associated with an increase in risk of prostate cancer in older men, suggesting that vitamin D has a protective role against prostate cancer mainly before the andropause, when serum androgen concentrations are higher (90).

Synthetic Vitamin D Analogues

Many analogues of vitamin D have been developed as candidates for clinical use. Most of these mimic the anticarcinogenic effects of vitamin D without appreciably affecting calcium metabolism. Clinical uses of noncalcemic vitamin D analogues include pro-differentiating effects that are potentially useful against some forms of cancer, such as RO 24-5531, a fluorinated analog of 1,25(OH)2D (171). There was a 70% reduction in incidence of colon tumors in animals given RO 24-5531 compared with controls (171). None of the tumors in the treated group were malignant, while 40% in the control group were malignant. The malignant tumors in the control group were adenocarcinomas, while the treated group experienced only benign adenomas (171). This indicates that vitamin D analogues are able to prevent progression from adenomas to malignancies in the colon.

Another vitamin D analog, RO 25-6760 (16-ene-23yne-26, 27-hexafluoro-19-nor-$1,25(OH)D_3$), has been shown to have an antimitogenic action five times greater than that of $1,25(OH)_2D$ (172). The effects of 1,25-dihydroxyvitamin D_3 and RO 25-6760 have been evaluated in vitro and in vivo in human colorectal cancer with high (HT-29) and low (SW-620) levels of vitamin D receptor. RO 25-6760 and $1,25(OH)_2D$ caused growth inhibition of both

cell lines, with a dose gradient (172). Another analog, 24R,25-dihydroxyvitamin D_3 inhibits rat colon cancer development during the post initiation period (173). Both $1,25(OH)_2D$ and a synthetic analog reduce intestinal tumor load in the Apc (min) mouse (28).

Treatment of breast cancer (MCF-7) cells with $1,25(OH)_2D_3$ reduces estrogen receptor levels in a dose-dependent manner (174). Vitamin D analogues EB1089, KH-1060, RO 27-0574, and RO 23-7553 are more potent than $1,25(OH)_2D_3$ in antiproliferative actions and estrogen receptor down-regulation (174). The vitamin D analog EB1089 (22,24-diene-24a, 26a, 27a-trihomo-$1,25(OH)_2D_3$ increases the rate of apoptosis sixfold in breast cancer cell cultures treated with adriamycin (11), suggesting a role in chemotherapy for vitamin D analogues. EB1089 also unexpectedly enhances the response of human breast cancer cells to therapeutic radiation (175) and produces regression of prostatic cells (176). The effect on breast cancer cells is mainly due to enhancement of induction of apoptosis of the tumor cells (177). The analog RO 25-6760 produced a dose-dependent growth inhibition of breast cancer cell lines (MCF-7 and MDAMB-468) (178) and counteracted growth stimulation of breast cancer cells by estradiol, TGF-a and IGF-I, and blocked progesterone receptor induction by estradiol (178). EB1089 also has been shown to be effective in controlling growth of breast cancer cell lines that have become resistant to tamoxifen (179). This is an active area of investigation, and new noncalcemic analogues of $1,25(OH)_2D$ are currently in development as potential adjunctive agents in cancer treatment. Vitamin D receptor levels usually are upregulated in human breast cancer cells (180), suggesting that breast cancer may be amenable to adjunctive approaches with vitamin D analogs or metabolites.

Prevalence of Vitamin D Deficiency

Vitamin D deficiency is common in the winter in the United States (181) and Europe (64). The vitamin is available from only a few sources (Fig. 6). Urban lifestyle has reduced average exposure of adults to midday sunlight to a low level except during summer months (64, 182, 183). Vitamin D that is photosynthesized in summer does not persist through the winter (66, 182-186). Vitamin D is metabolized within 3-5 days to 25(OH)D, the storage form (182), and the half-life of 25(OH)D is between 12 days (184) and 27 days (185).

A study of patients admitted to general hospitals found that 57% were seriously vitamin D-deficient (serum concentration of 25(OH)D $\leq$15 ng/ml), of whom 22% were considered severely vitamin D-deficient (serum concentration of 25(OH)D <8 ng/ml) (187); similar

deficiency states were reported in New England women (188, 189) and in black women (82, 190-193). Vitamin D deficiency is extremely prevalent in obese women, whose 25(OH)D level tends to be markedly below normal (191, 194) and whose serum 25(OH)D increases only slightly after UVB irradiation or oral intake of vitamin D (194). Serum 25(OH)D levels are, of course, also markedly low in women with hip fractures (195). Serum 25(OH)D is not well restored seasonally in black women (196); in one recent study, 42% of black women ages 15-49 years were found to have seriously deficient 25(OH)D levels (below 15 ng/ml) (193). Serum 25(OH)D levels in the range of 20-32 ng/ml are minimally required for parathyroid hormone levels to plateau at normal values (64, 197).

The vitamin D deficiency state is defined by measurement of the serum 25(OH)D level (181, 195, 198). Relatively inexpensive, reliable clinical tests for 25(OH)D in serum are readily available through virtually all major clinical laboratories. The previously recognized normal range for prevention of osteomalacia due to vitamin D deficiency has been 15-55 ng/ml. More recent research suggests that the threshold for prevention of osteomalacia is higher, reaching to 20 ng/ml (199) or 30 ng/ml (200, 201). Serum 25(OH)D levels below 30 ng/ml also predispose to colorectal (84, 85) and breast cancer (5), and may be associated with increased incidence of prostate cancer (90). 25(OH)D levels above 150 ng/ml, by contrast, are indicative of potential toxicity (202-204).

Toxicity

Vitamin D is stored in fat and other tissues and therefore can have toxic effects when administered in large doses on a persistent basis (205). The National Academy of Sciences—Institute of Medicine, has suggested 2,000 IU per day as the safe upper limit of vitamin D intake for children over one year old and adults (206). This recommendation is considered conservative (207), but recommended daily oral intakes are usually well below this level. The recommended daily intake is 200 IU (5 µg) at ages 1-50 years, 400 IU (10 µg) at ages 51-70 years, and 600 IU (15 µg) at age 71 years and older (206, 208). Vitamin D_3 raises serum 25(OH)D more efficiently than vitamin D_2 (209). Potential toxic effects such as bone demineralization, hypercalcemia, hypercalciuria or nephrocalcinosis with renal failure are encountered rarely, and, generally, only when the daily dose exceeds 10,000 IU on a chronic basis (210). Concerns about vitamin D toxicity in the past have been due to effects of massive overdoses in the range of 50,000-150,000 IU per day on a relatively long-term basis (59, 203). No major health risks are currently known to be associated with dosages of vitamin D in the

normal range of intake (up to 2,000 IU per day) (204,206, 207, 211, 212). Serum 25(OH)D in the normal range is not associated with any adverse risk of cardiovascular disease (213, 214). On the contrary, above-average serum levels of 25(OH)D and vitamin D intake are associated with reduced blood pressure (215-217) and heart rate (217), which are both favorably associated with risk of heart disease (215-217). Above-average 25(OH)D levels are also associated with lower risk of myocardial infarction (192, 213, 214). Daily intake of 600-800 IU of vitamin D_3 will maintain serum 25(OH)D in the range for its anticarcinogenic effect, without known risk of toxicity (64).

Genetic Factors

Most actions of vitamin D metabolites are mediated by the VDR. Mice genetically lacking the classical nuclear VDR have poor skeletal mineralization and a markedly higher rate of colonic proliferation than wild-type mice (218). Gene loci related to the receptor in humans have several common polymorphisms (219). Those most closely associated with cancer risk are discussed here. The Bsm I locus is most related to risk of cancer (220-222). It has three polymorphisms: BB, Bb, and bb. The BB genotype is associated with higher circulating $1,25(OH)_2D$ levels (220).

A cohort study of physicians found that doctors with the BB genotype had half the risk of colon cancer (OR, 0.5, 95% CI, 0.3-0.9) (220) as those with the bb genotype. The polyA (short), and TaqI (tt) variants of the VDR gene also have been found to be associated with reduced risk of colon cancer, while the Fok I VDR variant is not associated with risk (221).

A study of women found that those with the BB genotype had less than half the risk of breast cancer (222) as those with the bb genotype. As with colon cancer, the Fok I genotype was not associated with risk of breast cancer (222, 223). Polymorphisms of the VDR ApaI VDR locus also are associated with risk of breast cancer; women with the AA genotype have three times the risk of breast cancer as those with the Aa genotype (224). Allele frequencies of the Fok I polymorphism were not significantly different in breast cancer cases than controls (223) .

Physicians with the BB genotype had lower risk of prostate cancer than those with the bb genotype (220). The effect was strongest in physicians whose serum 25(OH)D level was below the median (OR, 0.43) and in older physicians (OR, 0.18). The gene frequency of the BB polymorphism is estimated to be 8-10% (224, 225). In another study, having the Bb or bb Bsm I

polymorphism was associated with having three times the risk of prostate cancer (226). A race-adjusted analysis in an earlier study found that men who were homozygous for the t allele of the Taq I VDR gene (codon 352) (for which homozygosity was previously known to associated with higher than average serum levels of $1,25(OH)_2D$ (227)) had one third the risk of developing prostate cancer requiring prostatectomy than men who were heterozygous or homozygous for the T allele (228). A European study found that men with no T alleles had half the risk of prostate cancer compared with those with one or more T alleles (229). Since the T polymorphism is common, approximately 50% of cases of prostate cancer in men older than 66 years in one population studied in Europe could be attributed to the effect of this polymorphism (229). In another study, the genotype TT at the Taq I locus was significantly associated (OR, 5.4) with having a high Gleason grade of tumor (grade 5) (230). While other studies have been inconclusive regarding the association of specific VDR polymorphisms with prostate cancer (231, 232), individuals with non-BB genotypes at the Bsm I site or who have one or more T alleles at the Taq I VDR site may require more vitamin D intake for reduction of risk of prostate cancer than those with other genotypes.

Recommendations for Vitamin D Intake

Vitamin D deficiency is highly prevalent in North America (181, 186, 193). This deficiency is due to inadequate dietary vitamin D intake and limited solar UVB exposure, especially during winter months (15, 182). Daily intakes of vitamin D of no less than 200-400 IU at ages 1-49 years, 400 IU at ages 50-70 years, and 600 IU at ages 71 years and older constitute the minimum dietary intakes necessary to counteract these deficiencies with regard to osteomalacia, assuming normal absorption of vitamin D. These minimal intakes would also be desirable for reducing the incidence of colon, breast, prostate, ovarian, and possibly other cancers.

Exposure to the sun during November through March in the northeastern United States does not allow any synthesis of vitamin D in the skin (53), and serum levels of vitamin D metabolites that are needed to prevent many important diseases typically cannot be maintained throughout winter. This is especially true in older persons, who cannot synthesize vitamin D as well as that of younger persons in response to ultraviolet light (17, 66) and whose vitamin D from diet is less readily absorbed in the intestine (233). It has been estimated the vitamin D

deficiency may account for more than 20,000 premature deaths from cancer annually in the U.S. (43).

When there is any doubt regarding vitamin D status, it should be tested using a simple, reliable, and widely available clinical laboratory test for serum 25(OH)D (183). The test preferably should be performed in the Fall, if possible, to identify the risk of vitamin D deficiency during the winter months. The target range of 30-50 ng/ml of serum 25(OH)D that is needed to prevent osteomalacia and fractures (200, 201) is the same as the target range for reduction of the incidence of colon, breast, prostate, and possibly other cancers throughout life.

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Figure Legends

Figure 1. Data are accumulating that support a mechanism for vitamin D in reducing the risk of malignancy. The effect is based on the known role that intracellular Ca^{2+} plays in differentiation and apoptosis, and is based on research by Davies and colleagues (92). It is built on the existence of an intracellular Ca^{2+} gradient from the base to the top of the colonic crypts that was first described by Lipkin and colleagues (3, 99). This figure shows Ca^{2+} channels in the colonocyte membrane that function as receptors for Ca^{2+}, allowing entry of vitamin D metabolites that are thought to exist in the cell membrane, and calbindin molecules that are synthesized in the cell in response to stimulation of the nuclear vitamin D receptor by $1,25(OH)_2D$ (234). Each calbindin molecule reversibly binds four Ca^{2+} ions, making them available for pulsatile release when needed for intracellular physiological roles, including differentiation and apoptosis. If intracellular vitamin D metabolites are deficient, calbindin is virtually absent from the cytoplasm. As a result, rapid pulsatile release of Ca^{2+} is impossible at the level needed to sustain differentiation and apoptosis. This may be because there is an inadequate concentration of the intracellular calbindin molecules that normally serve as Ca^{2+} "capacitors" and rapidly release Ca^{2+} ions for these roles.

Figure 2. Normal structure and pattern of differentiation and apoptosis in colonocytes, based on the concept developed by Lipkin and colleagues (3, 99). As the colonocytes migrate from the base of the crypt to the top, they differentiate and ultimately undergo apoptosis. These functions are mediated by the intracellular Ca^{2+} concentration, which increases under normal conditions as the cells migrate upward. There is also a gradient in the lumenal Ca^{2+} concentration that is thought to contribute to differentiation and apoptosis at the top of the crypt if the lumenal contents are replete with Ca^{2+}.

Figure 3. Colonic crypt in the vitamin D-replete individual. In the cell of a vitamin –D-replete individual, there is a strong gradient in the intracellular Ca^{2+} from a low concentration at the base to a high concentration at the top (91). Calbindin provides a means of storing and releasing Ca^{2+}, and it is thought to mediate the intracellular Ca^{2+} gradient from the low concentration at the base of the crypt to the usually high concentration at the top. When $1,25(OH)_2D$ is abundant in the colonocyte, a high concentration of calbindin is produced (97). The intracellular free Ca^{2+}

ions are needed for terminal differentiation and, ultimately, apoptosis in the vitamin D-replete cell (91).

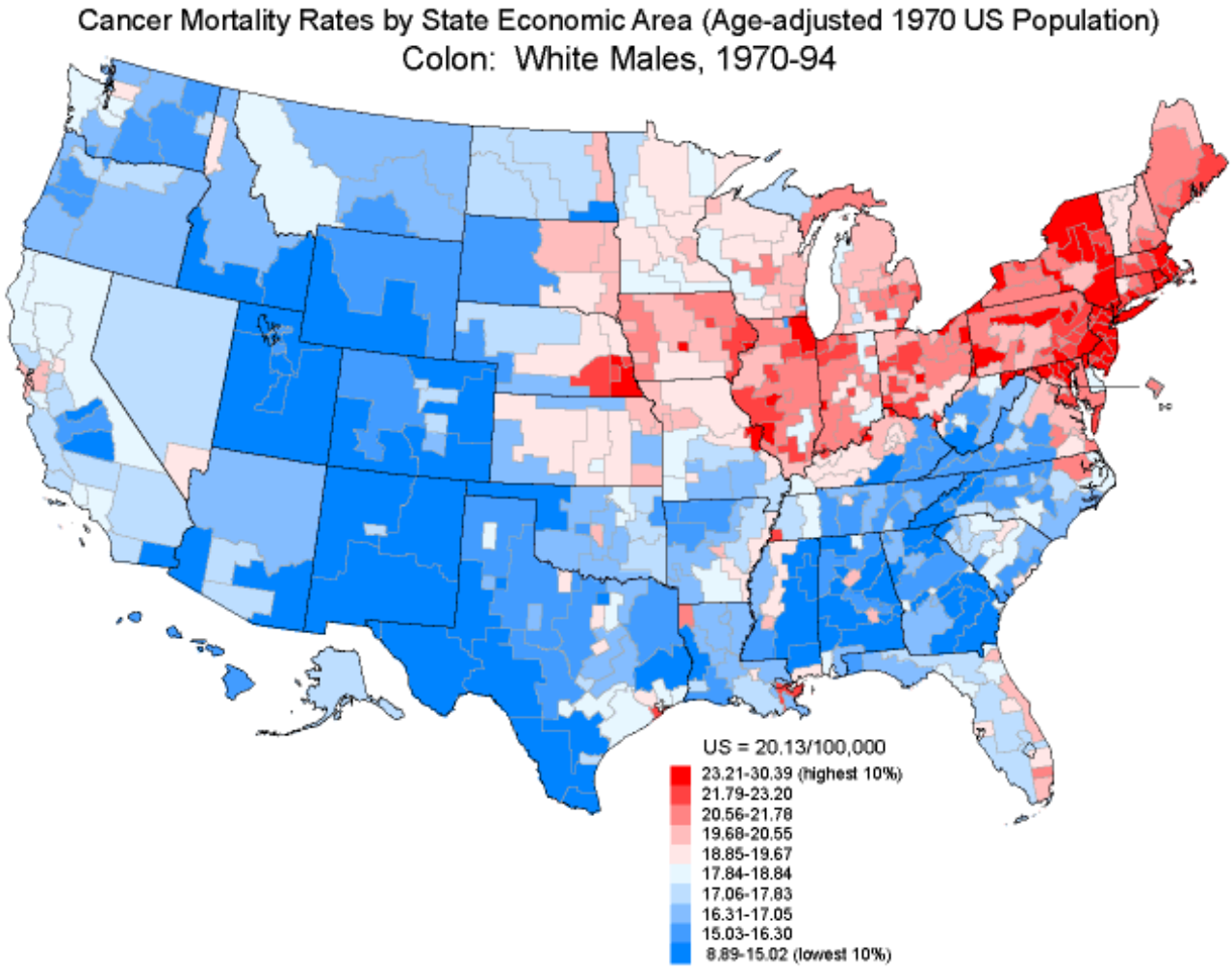
Figure 4. Colonic crypt in the vitamin D-deficient individual (91). When vitamin D metabolites are deficient in the cell, calbindin is present in low concentration or nonexistent. There are no means of reversibly storing and rapidly releasing the Ca^{2+} that mediates the intracellular Ca^{2+} gradient from the base of the crypt to the mouth. When this gradient is absent, the colonocytes do not terminally differentiate as they migrate up the crypt, and the normal occurrence of apoptosis at the mouth of the crypt is inhibited or prevented (3, 99). An adenomatous polyp results from buildup of senescent colonocytes that have not undergone apoptosis, and ultimately a cancer may evolve in the polyp or nearby if other conditions are appropriate. The effect of loss of the intracellular Ca^{2+} gradient is thought to be exaggerated when the lumenal Ca^{2+} concentration is low. Other genomic and nongenomic mechanisms for the anticarcinogenic role of vitamin D in colonocytes have also been described (109-111, 235-238).

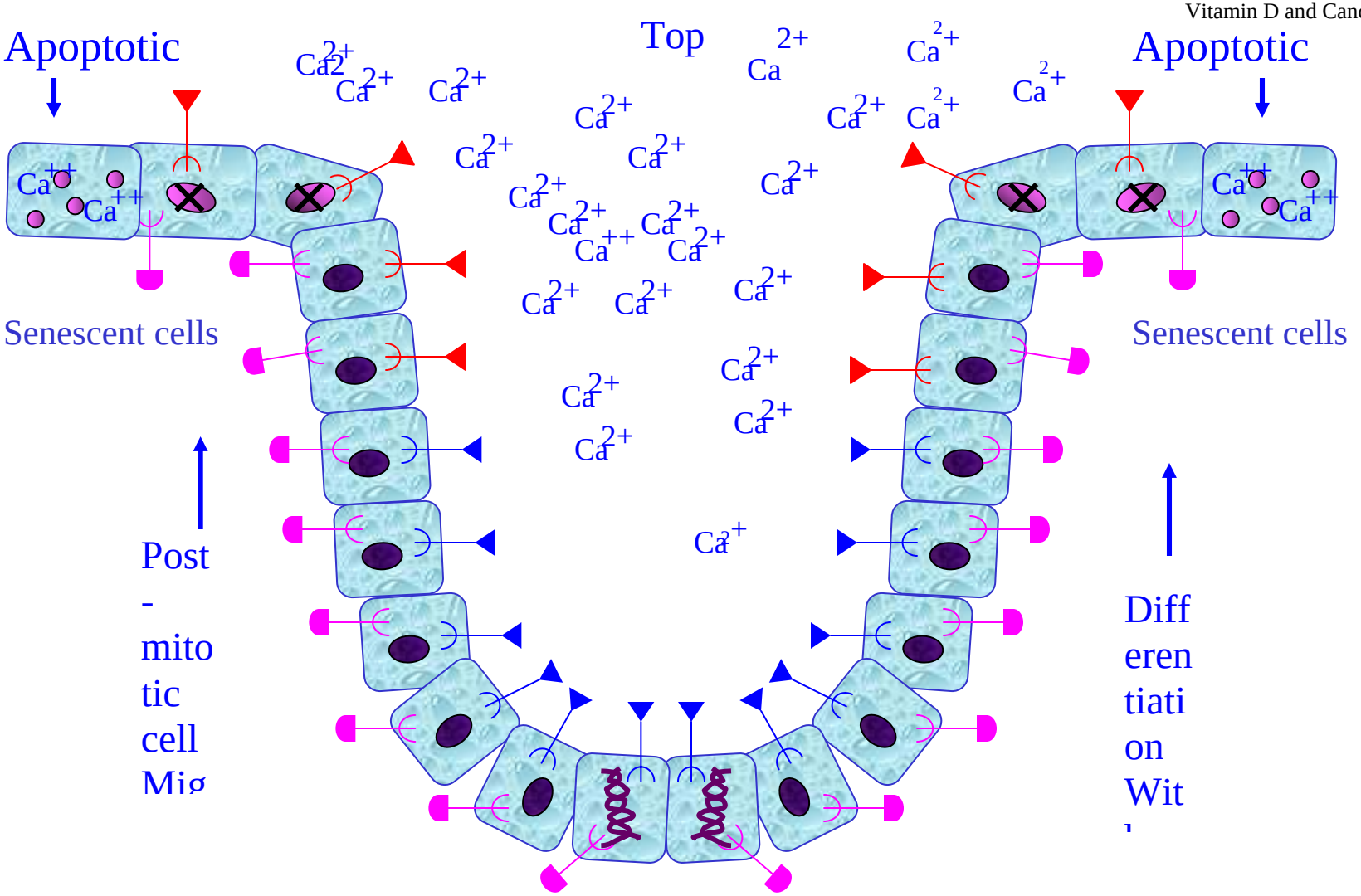
Figure 5. Colon cancer mortality rates are uniformly low (blue tints) south of about 37 degrees latitude in the United States, especially in the sunniest areas (New Mexico, Western Texas, Utah, and Arizona). By contrast, rates are highest (pink and red tints) in areas that receive the least solar ultraviolet B (UVB), including New York, New Jersey, Pennsylvania, and parts of New England. Winter UVB radiation levels in the Northeastern industrial sector are so low that no vitamin D can be photosynthesized during November through February (53). The low UVB levels are due to climate (cold and cloudy arctic and Canadian air masses transported to the region during the late fall and winter), relatively high latitude, removal of UVB from sunlight by a type of air pollution (acid haze) that predominates in the region (86, 87), and a thick stratospheric ozone layer that exists over the Northeastern sector of the United States (86). Death rates in the areas with the highest rates are approximately twice those in areas with the lowest rates. (Source: National Cancer Institute, 2002. Rates shown are age-adjusted mortality rates for white men during 1970-1994. The pattern of rates was virtually identical for white women.)

Figure 6. Vitamin D deficiency is common in the winter in North America (180, 181) because the vitamin is available from only a few sources. Urban lifestyle has reduced average exposure of

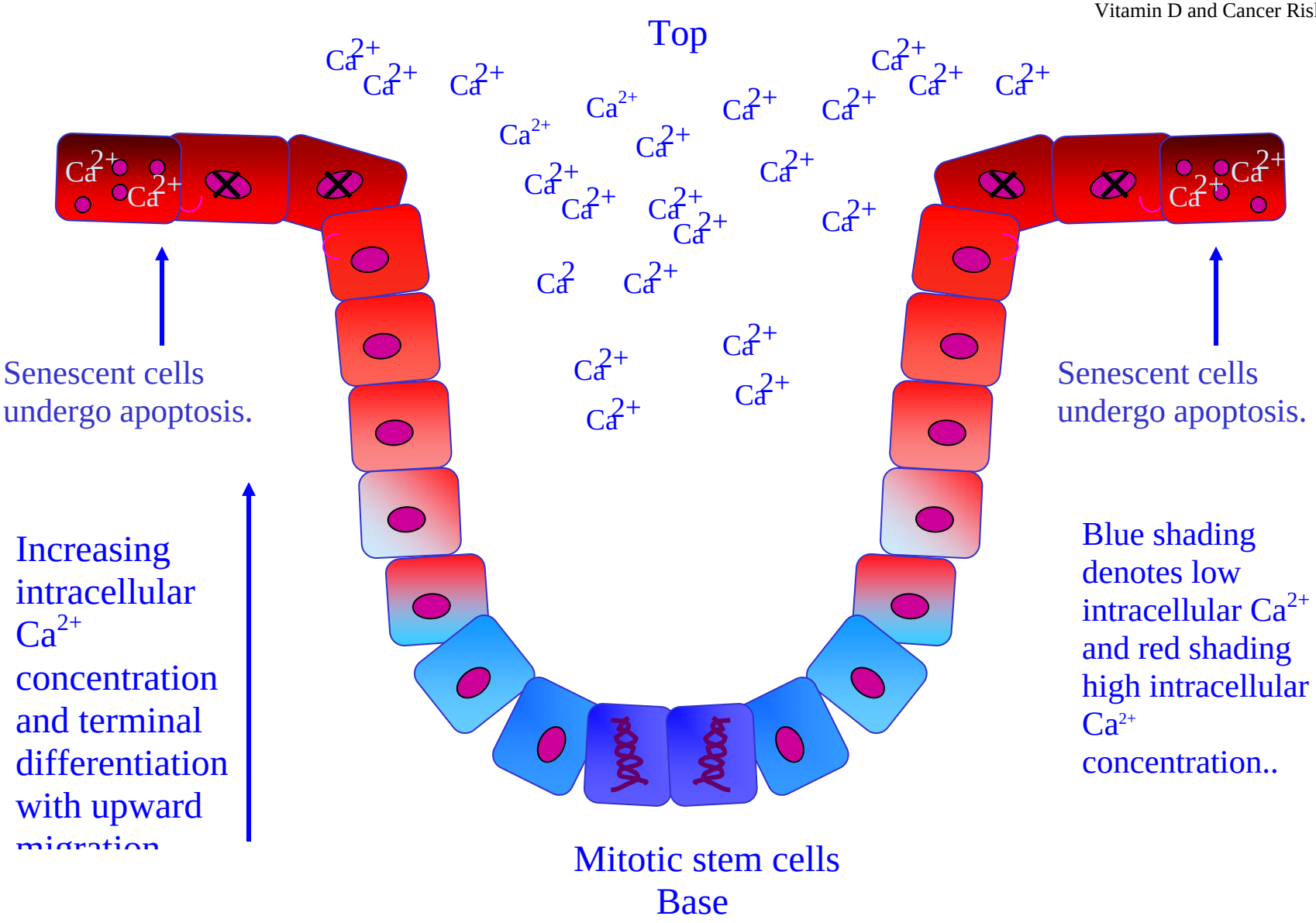
adults to midday sunlight to a low level except during summer months. Vitamin D that is photosynthesized in the summer does not persist through the winter (66, 182, 184-186). Vitamin D is metabolized within 3-5 days to 25(OH)D, the storage form (182). The half-life of 25(OH)D is between 12 days (184) and 27 days (185).

Figure 5. (Figures 1-4 and 6 are in a PowerPoint file (attached) named VitDMech_Figs1-4_&_6_TRv3_REVISED.ppt

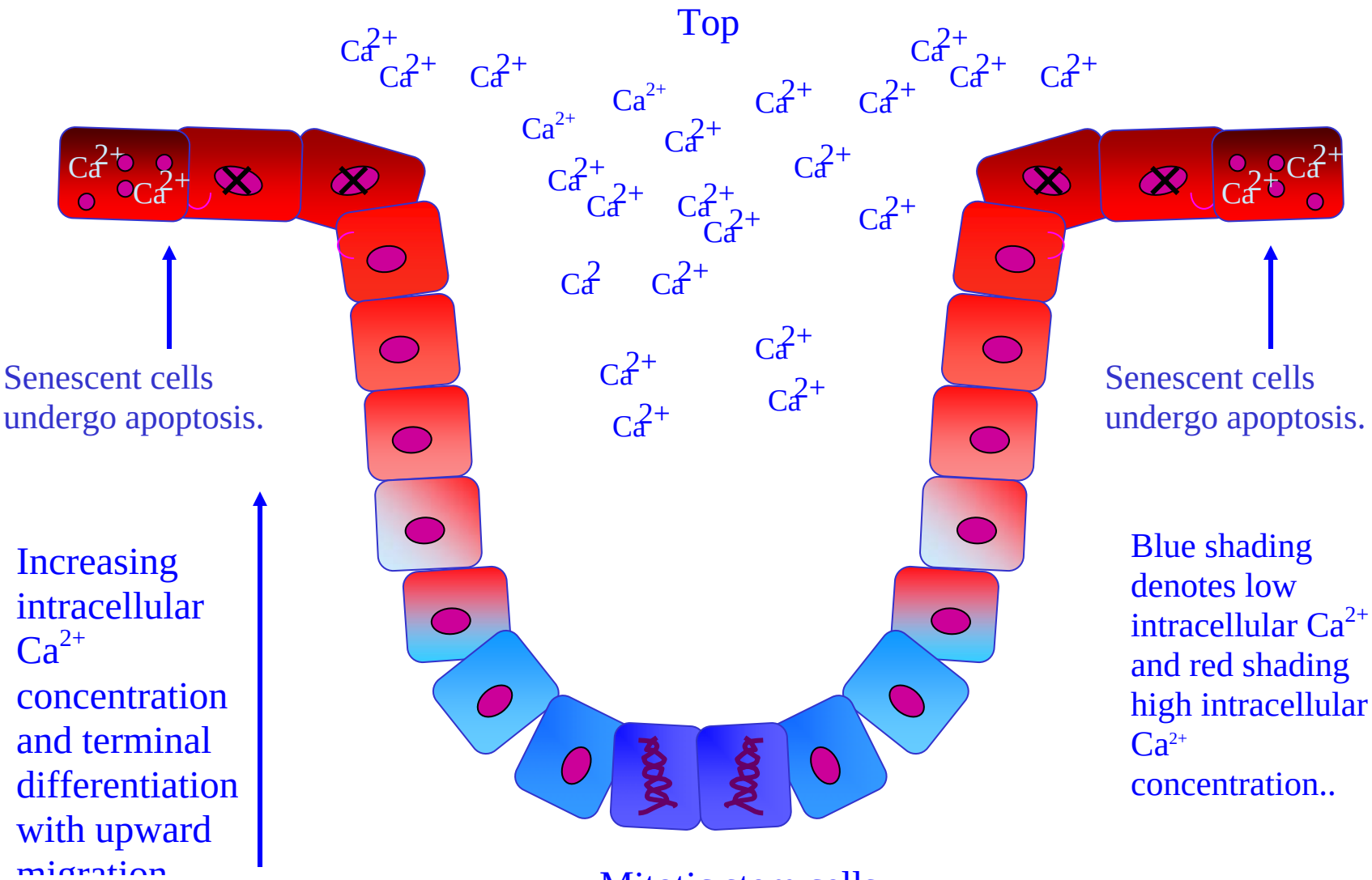




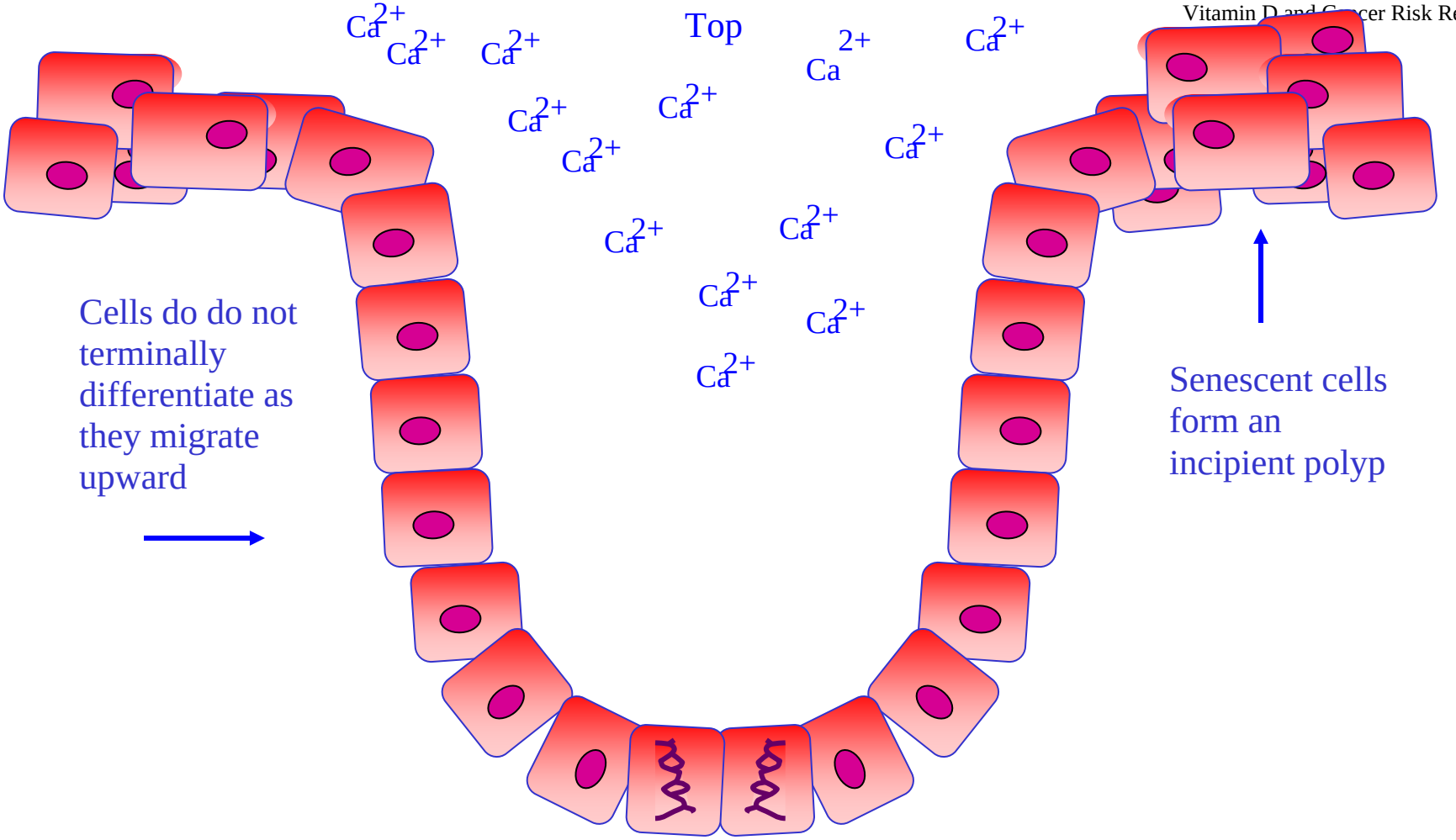
Normal colonic crypt differentiation and



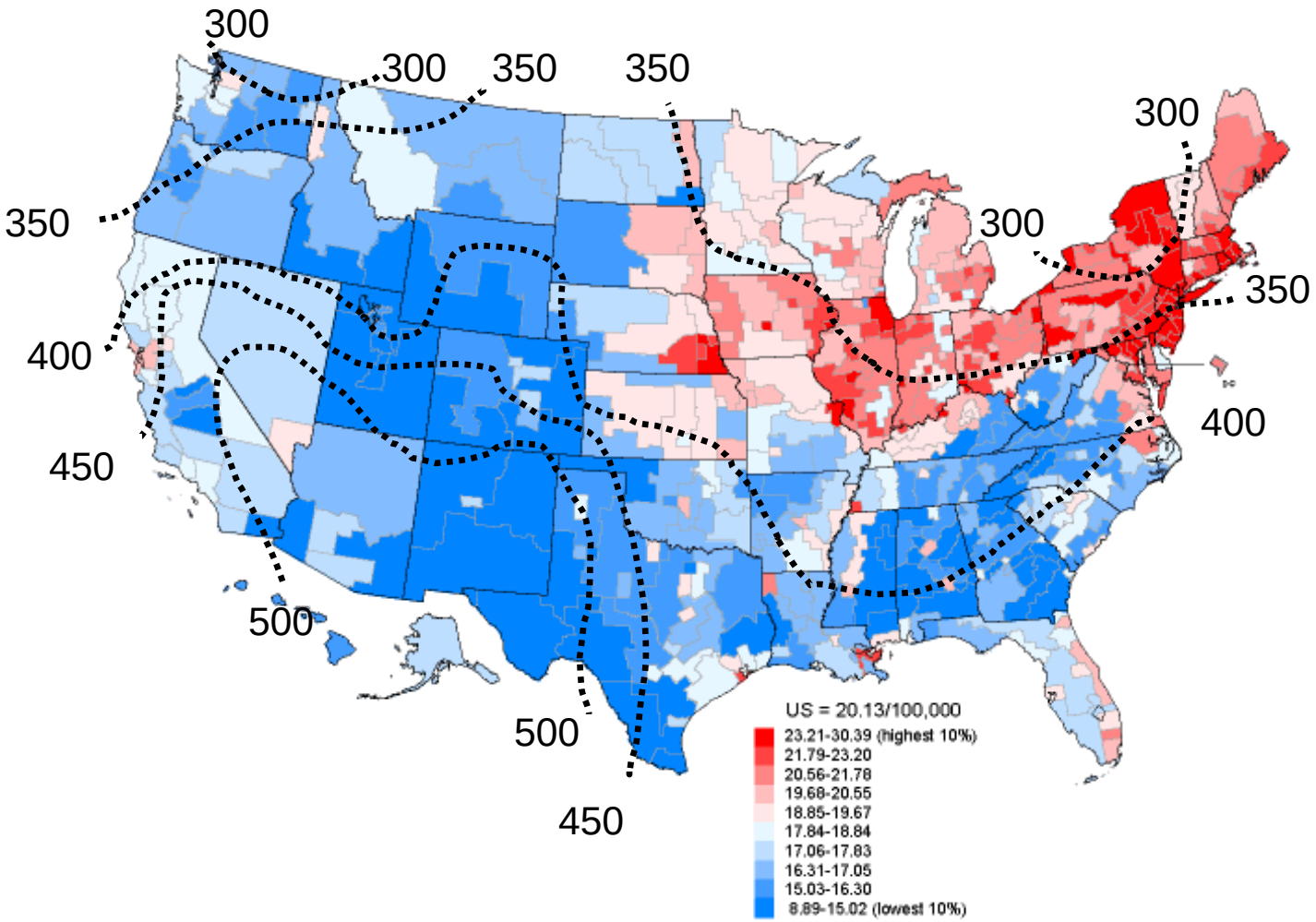
Vitamin D replete crypt: intracellular Ca^{2+} concentration gradient is

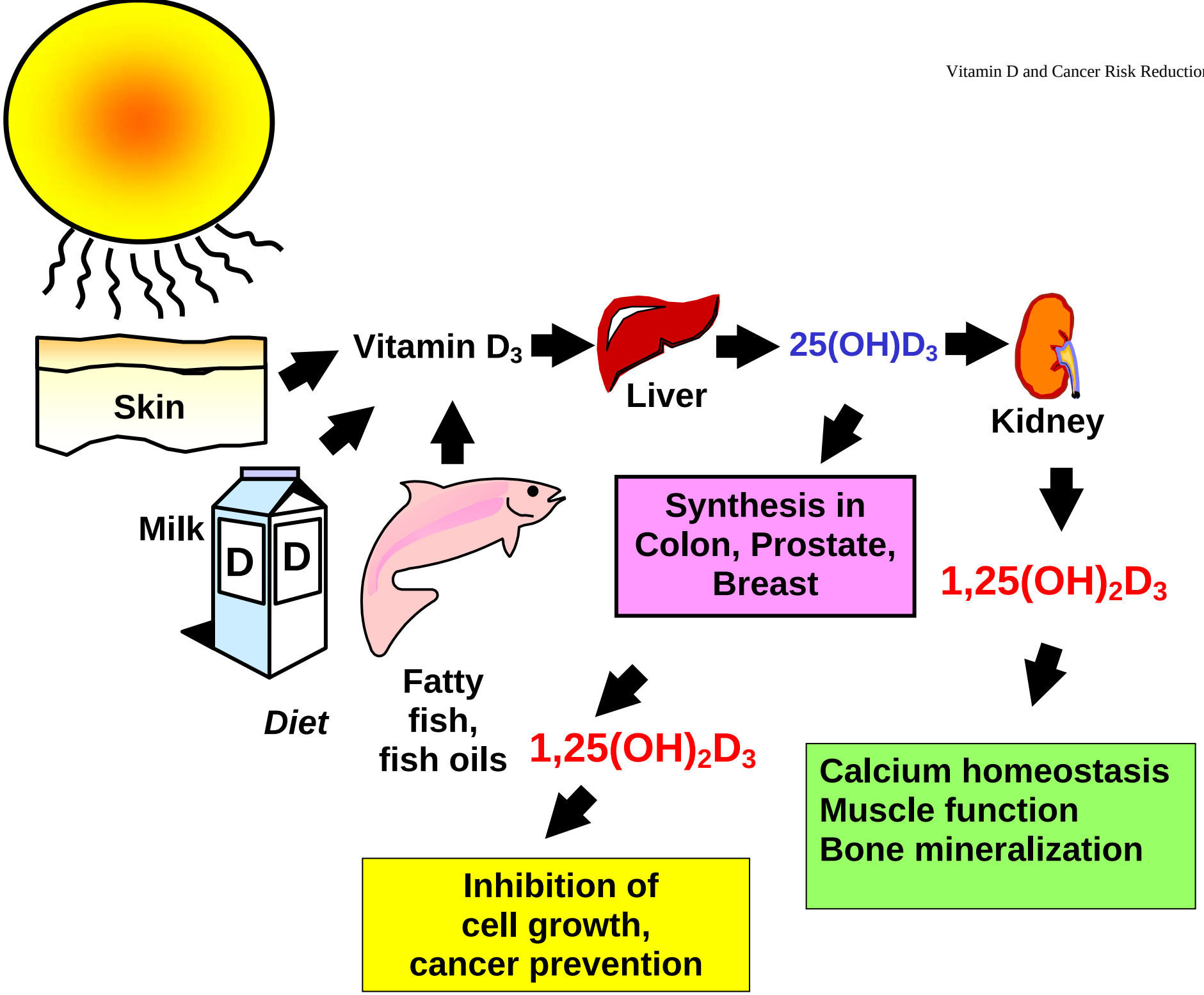


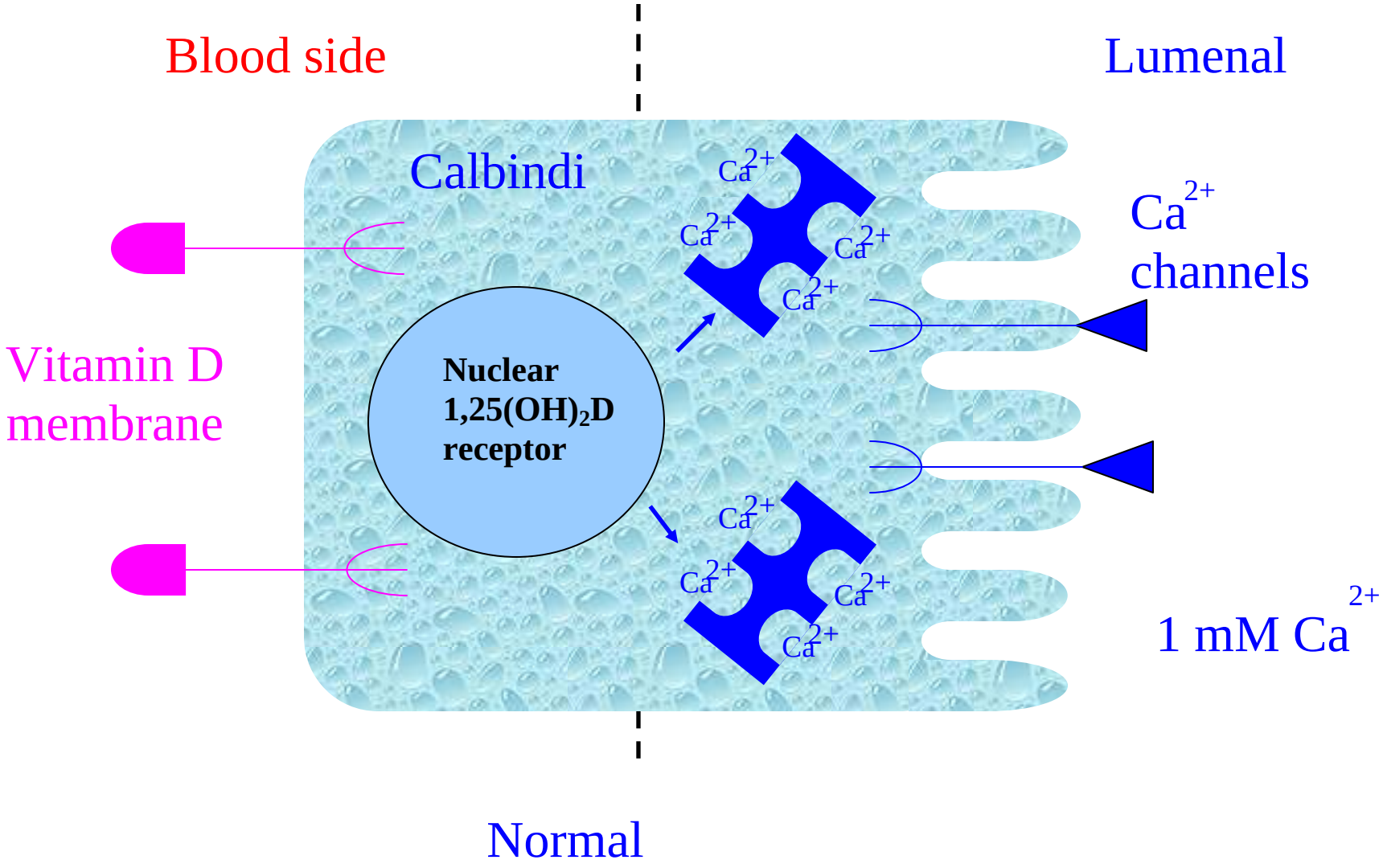
Vitamin D replete crypt: intracellular Ca^{2+} concentration gradient is



Vitamin D deficient crypt: intracellular Ca^{2+} concentration gradient is abolished


300
300
350
350
300
350
300
350
400
450
400
500
500
450
US = 20.13/100,000
23.21-30.39 (highest 10%)
21.79-23.20
20.56-21.78
19.68-20.55
18.85-19.67
17.84-18.84
17.06-17.83
16.31-17.05
15.03-16.30
8.89-15.02 (lowest 10%)


Skin
Vitamin D_3
Liver
$25(OH)D_3$
Kidney
Milk
D
D
Diet
Fatty fish, fish oils
Synthesis in Colon, Prostate, Breast
$1,25(OH)_2D_3$
$1,25(OH)_2D_3$
Calcium homeostasis
Muscle function
Bone mineralization
Inhibition of cell growth, cancer prevention


Blood side
Lumenal
Calbindi
Ca2+
Ca2+
Ca2+
Ca2+
Ca2+
Ca2+
Ca2+
Ca2+
Ca2+
Ca2+
Vitamin D membrane
Nuclear 1,25(OH)2D receptor
Ca2+ channels
1 mM Ca2+
Normal